JC07 Rec'd PCT/PTO 07 DEC 2001

FORM PTO-1390 (Modified) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE
(REV 5-93)

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 065691-0260

U.S. APPLICATION NO. (If known, see 37 CFR 1.5): Unassigned 09/980833

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/FR00/01560	06/07/2000	06/07/1999

TITLE OF INVENTION
Novel Ig Fractions Having Immunomodulatory Activity

APPLICANT(S) FOR DO/EO/US
Dominique Bourel et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   - ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   - ☒ has been transmitted by the International Bureau.
   - ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   - ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   - ☐ have been transmitted by the International Bureau.
   - ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   - ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).
11. ☐ Applicant claims small entity status under 37 CFR 1.27 .

Items 12. to 17. below concern other document(s) or information included:

12. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
13. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
14. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☒ Other items or information: Copy of International Search Report; Verification of English Translation

JC10 Rec'd PCT/PTO 07 DEC 2001

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
Unassigned 09/980833		065691-0260

18. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5): Search Report has been prepared by the EPO or JPO $890.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) $710.00		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2) $740.00		
Neither international preliminary examination fee (37 CFR 1.482) nor International search fee (37 CFR 1.445(a)(2)) paid to USPTO $1,040.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) $100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$890.00	
Surcharge of $130.00 for furnishing the oath or declaration later than 20 Months from the earliest claimed priority date (37 CFR 1.492(e))	$130.00	

Claims	Number Filed		Included in Basic Fee		Extra Claims		Rate		
Total Claims	29	-	20	=	9	x	$18.00	$162.00	
Independent Claims	2	-	3	=	0	x	$84.00	$0.00	
Multiple dependent claim(s) (if applicable)							$280.00		
TOTAL OF ABOVE CALCULATIONS =								$1182.00	
Reduction by ½ for filing by small entity, if applicable.								$0.00	
SUBTOTAL =								$1182.00	
Processing fee of $130.00 for furnishing English translation later the 20 months from the earliest claimed priority date (37 CFR 1.492(f). +								$ 130.00	
TOTAL NATIONAL FEE =								$1312.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +									
TOTAL FEES ENCLOSED =								$1312.00	
							Amount to be: refunded	$	
							charged	$	

a. ☒ A check in the amount of $1312.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. 19-0741 in the amount of -$ 0 - to the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 19-0741. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Foley & Lardner
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22428

PATENT TRADEMARK OFFICE

SIGNATURE Michael D. Kaminski
Reg. No. 32,904, for

NAME STEPHEN B. MAEBIUS

REGISTRATION NUMBER 35,264

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Dominique Bourel et al.

Entitled: Novel Ig Fractions Having Immunomodulatory Activity

Serial No.: To be assigned

Date Filed: Concurrently

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination of the present application, Applicant's respectfully requests that the above-identified application be amended as follows:

In the Claims:

In accordance with 37 C.F.R. § 1.121(c) (3), please substitute for pending claims 3, 4, 6-9, 12, 14, 15-23, 25, and 26 with the following clean version of the claims. The changes to these claims are shown explicitly in the attached "Marked Up Version of Claims."

3. (Amended) The fraction as claimed in 1, characterized in that it reacts with a component selected from IgMs, IgG F(ab')2s and the hapten DNP, with a level of enrichment of greater than 40 compared to the activity of the initial polyvalent Igs.

4. (Amended) The fraction as claimed in claim 1, characterized in that it reacts with at least one of the autoantigens selected from myosin, actin, tubulin and myelin basic protein (MBP), with a level of enrichment of greater than 10 compared to the activity of the initial polyvalent Igs.

6. (Amended) The fraction as claimed in claim 1, characterized in that it reacts with myosin, actin, tubulin and MBP.

7. (Amended) The fraction as claimed in claim 1, characterized in that it reacts with a component selected from IgMs, IgG F(ab')2s and the hapten DNP, with a level of enrichment of greater than 40 compared to the activity of the initial polyvalent Igs, and with myosin, actin, tubulin and MBP, with a mean level of enrichment of greater than 20 compared to the activity of the initial polyvalent Igs.

8. (Amended) The fraction as claimed in claim 1, characterized in that it reacts with IgMs or IgG F(ab')2s.

9. (Amended) The fraction as claimed in claim 1, characterized in that it reacts with the hapten DNP and in that it does not react with IgMs and IgG F(ab')2s.

12. (Amended) The method as claimed in claim 10, characterized in that the Ig fractions are prepared from polyvalent Igs or any other intermediate fraction obtained during the method for producing IVIgs for therapeutic use.

14. (Amended) The method as claimed in claim 10, characterized in that step d) comprises measuring the level of enrichment of antibodies reactive against IgMs, IgG F(ab')2s or the hapten DNP used for the purification.

15. (Amended) The method as claimed in claim 10, characterized in that step d) comprises measuring the reactivity for the tetanus toxoid and the HBs antigen, taking the level of enrichment as a control value.

16. (Amended) The method as claimed in claim 10, characterized in that step d) comprises an ELISA assay carried out on a panel of autoantigens selected in particular from actin, myosin, MBP and tubulin.

17. (Amended) The method as claimed in claim 10, characterized in that step d) comprises a competition assay in order to control the neutralizing activity of the

fractions with respect to autoantibodies originating from serum of patients suffering from autoimmune diseases.

18. (Amended) The method as claimed in claim 10, characterized in that step d) comprises an assay of inhibition of the mixed lymphocyte reaction with human cells in order to control the reactivity of the purified Igs.

19. (Amended) The method as claimed in claim 10, characterized in that step a) consists in grafting polyvalent IgGs, polyvalent IgMs or DNP-Lysine onto an insoluble support, in particular onto a Sepharose®, Trisacryl®, Affiprep® or Affigel® gel, or gels activated with the groups CNBr, NHS or $C_5H_8O_2$ (glutaraldehyde).

20. (Amended) The method as claimed in claim 10, characterized in that the Igs deposited onto the solid support obtained in step a) are adsorbed either in the form of polyvalent IgGs lyophilized and redissolved or in liquid form, or in the form of intermediate fractions obtained during a method for producing polyvalent IgGs, in 20 mM phosphate buffer containing NaCl, the concentration of which may range from 0 M to 3 M.

21. (Amended) The method as claimed in claim 10, characterized in that the Igs retained in step b) are eluted with a buffer containing ions which dissociate Ag-Ab or Ag-DNP binding, selected in particular from chaotropes such as glycine-HCl or sodium iodide (NaI), under conditions which vary the pH, preferably between 2.8 and 4.0, and/or the molarity of the buffer.

22. (Amended) The method as claimed in claim 10, characterized in that the absorption is carried out under temperature conditions ranging from 4° to 40°C and in PBS.

23. (Amended) The method as claimed in claim 10, characterized in that, in step d), fractions characterized in that it reacts with at least one component selected from IgMs, IgG F(ab')2s and the hapten DNP, with a level of enrichment of greater than 20 compared to the activity of the initial polyvalent Igs, and in that it does not react

with the tetanus toxoid or the HBs antigen, with a level of enrichment of less than 5 compared to the activity of the initial polyvalent Igs is selected.

25. (Amended) A fraction which can be obtained using a method as claimed in claim 10.

26. (Amended) The use of an Ig fraction as claimed in claim 1, for preparing a medicinal product.

REMARKS

Applicant respectfully requests that the foregoing amendments be made prior to examination of the present application.

Respectfully submitted,

Date December 7, 2001

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MARKED UP VERSION OF AMENDED CLAIMS

3. (Amended) The fraction as claimed in [either of claims 1 and 2] claim 1, characterized in that it reacts with a component selected from IgMs, IgG F(ab')2s and the hapten DNP, with a level of enrichment of greater than 40 compared to the activity of the initial polyvalent Igs.

4. (Amended) The fraction as claimed in [one of claims 1 to 3] claim 1, characterized in that it reacts with at least one of the autoantigens selected from myosin, actin, tubulin and myelin basic protein (MBP), with a level of enrichment of greater than 10 compared to the activity of the initial polyvalent Igs.

6. (Amended) The fraction as claimed in [one of claims 1 to 5] claim 1, characterized in that it reacts with myosin, actin, tubulin and MBP.

7. (Amended) The fraction as claimed in [one of claims 1 to 6] claim 1, characterized in that it reacts with a component selected from IgMs, IgG F(ab')2s and the hapten DNP, with a level of enrichment of greater than 40 compared to the activity of the initial polyvalent Igs, and with myosin, actin, tubulin and MBP, with a mean level of enrichment of greater than 20 compared to the activity of the initial polyvalent Igs.

8. (Amended) The fraction as claimed in [one of claims 1 to 7] claim 1, characterized in that it reacts with IgMs or IgG F(ab')2s.

9. (Amended) The fraction as claimed in [one of claims 1 to 7] claim 1, characterized in that it reacts with the hapten DNP and in that it does not react with IgMs and IgG F(ab')2s.

12. (Amended) The method as claimed in [either of claims 10 and 11] claim 10, characterized in that the Ig fractions are prepared from polyvalent Igs or any other intermediate fraction obtained during the method for producing IVIgs for therapeutic use.

14. (Amended) The method as claimed in [one of claims 10 to 13] claim 10, characterized in that step d) comprises measuring the level of enrichment of antibodies reactive against IgMs, IgG F(ab')2s or the hapten DNP used for the purification.

15. (Amended) The method as claimed in [one of claims 10 to 14] claim 10, characterized in that step d) comprises measuring the reactivity for the tetanus toxoid and the HBs antigen, taking the level of enrichment as a control value.

16. (Amended) The method as claimed in [one of claims 10 to 15] claim 10, characterized in that step d) comprises an ELISA assay carried out on a panel of autoantigens selected in particular from actin, myosin, MBP and tubulin.

17. (Amended) The method as claimed in [one of claims 10 to 16] claim 10, characterized in that step d) comprises a competition assay in order to control the neutralizing activity of the fractions with respect to autoantibodies originating from serum of patients suffering from autoimmune diseases.

18. (Amended) The method as claimed in [one of claims 10 to 17] claim 10, characterized in that step d) comprises an assay of inhibition of the mixed lymphocyte reaction with human cells in order to control the reactivity of the purified Igs.

19. (Amended) The method as claimed in [one of claims 10 to 18] claim 10, characterized in that step a) consists in grafting polyvalent IgGs, polyvalent IgMs or DNP-Lysine onto an insoluble support, in particular onto a Sepharose®, Trisacryl®, Affiprep® or Affigel® gel, or gels activated with the groups CNBr, NHS or $C_5H_8O_2$ (glutaraldehyde).

20. (Amended) The method as claimed in [one of claims 10 to 19] claim 10, characterized in that the Igs deposited onto the solid support obtained in step a) are adsorbed either in the form of polyvalent IgGs lyophilized and redissolved or in liquid form, or in the form of intermediate fractions obtained during a method for

producing polyvalent IgGs, in 20 mM phosphate buffer containing NaCl, the concentration of which may range from 0 M to 3 M.

21. (Amended) The method as claimed in [one of claims 10 to 20] claim 10, characterized in that the Igs retained in step b) are eluted with a buffer containing ions which dissociate Ag-Ab or Ag-DNP binding, selected in particular from chaotropes such as glycine-HCl or sodium iodide (NaI), under conditions which vary the pH, preferably between 2.8 and 4.0, and/or the molarity of the buffer.

22. (Amended) The method as claimed in [one of claims 10 to 21] claim 10, characterized in that the absorption is carried out under temperature conditions ranging from 4° to 40°C and in PBS.

23. (Amended) The method as claimed in [one of claims 10 to 22] claim 10, characterized in that, in step d), fractions [as claimed in one of claims 1 to 9 are] characterized in that it reacts with at least one component selected from IgMs, IgG F(ab')2s and the hapten DNP, with a level of enrichment of greater than 20 compared to the activity of the initial polyvalent Igs, and in that it does not react with the tetanus toxoid or the HBs antigen, with a level of enrichment of less than 5 compared to the activity of the initial polyvalent Igs is selected.

25. (Amended) A fraction which can be obtained using a method as claimed in [one of claims 10 to 24] claim 10.

26. (Amended) The use of an Ig fraction as claimed in [one of claims 1 to 9 and 25] claim 1, for preparing a medicinal product.

NOVEL Ig FRACTIONS HAVING IMMUNOMODULATORY ACTIVITY

The present invention relates to a method for preparing Ig fractions from human polyvalent intravenous immunoglobulins (IVIgs) which are thought to be more particularly responsible for the immunomudulatory effect observed during treatments for certain autoimmune diseases. The invention relates to Ig fractions which have reactivity with respect to IgMs, IgG F(ab')2s or the hapten DNP, and little or no reactivity with respect to non-self antigens, i.e. fractions of Igs which exhibit interactions of the idiotypic type with one another (connected fraction) or which comprise natural antibodies which react with the hapten DNP. These fractions show polyreactivity with respect to given autoantigens.

IVIg preparations have been used for many years for treating multiple pathological conditions. The major indications may be grouped into three therapeutic targets:

- primary or secondary immune deficiencies,
- treatment of certain autoimmune diseases,
- infectious complications and graft-versus-host disease after allogenic hematopoietic cell transplants.

In the case of immune deficiencies, IVIgs constitute a substitutive treatment which makes it possible to provide IGgs, the plasma concentration of which in patients is not sufficient to neutralize the development of viral or bacterial infections.

For autoimmune diseases, the effectiveness of IVIgs is related to complex immunomodulatory effects. IVIgs are prescribed in the context of bone marrow transplants, and correspond to a substitutive treatment while

awaiting the immunological reconstitution of the individuals having received a transplant and exert an immunomodulatory effect with regard to graft-versus-host disease.

IVIgs are prepared from a pool of plasmas originating from several thousands of donors; they have a distribution of subclasses and antibody specificities reflecting that of the general population. Thus, IVIgs may be considered to be a product containing the entire repertoire of natural antibodies and of antibodies directed against outside antigens and autoantigens.

The concept of immunoregulation by IVIgs has been widely developed since the demonstration of their effectiveness in autoimmune thrombocytopenic purpura (AITP) in 1981 (1). IVIgs have subsequently been used in many autoimmune or inflammatory pathological conditions. Some indications, for which the effectiveness of IVIgs has been clearly established, are officially recognized by the regulating authorities. They are AITP, Kawasaki disease, in which they very effectively prevent complications concerning aneurysms (2, 3), allogenic hematopoietic cell transplantation, in which they modulate the graft-versus-host reaction (4) and, more recently, Birdshot retinochoroiditis, in which they improve visual acuity and in which they sometimes enable corticotherapy to be reduced (5).

Other indications are considered by experts to be justified; some cytopenias, for example, in which IVIgs lead to rapid but often transient improvement (6), and in hemophilias with inhibitors (anti-factor VIII autoantibodies), in which, on the other hand, the improvement may be long-lasting (7, 8). Contradictory results have been obtained in recurrent abortions, with encouraging success rates in certain series (9, 10).

For about ten years, there has been a very significant rapid development of IVIgs in neurology by virtue of controlled multicenter studies with both quantitative (neurological scores) and qualitative (number of patients improved) effectiveness criteria. Thus, in adult Guillain-Barré syndrome, IVIgs are as effective as plasma exchanges and are tolerated better (11, 12). They are recommended as first line treatment in pediatric forms (13). They are more effective versus placebo in chronic inflammatory demyelinating polyneuropathies (14) and in dermatomyositis (15). They are as effective as and better tolerated than plasma exchanges in acute episodes of myasthenia (16). Finally, a study versus placebo has demonstrated the effectiveness of IVIgs in relapsing/remitting forms of multiple sclerosis (17).

Several mechanisms have been proposed to explain the diversity of action of IVIgs (18):

- blocking of Fc receptors at the surface of macrophages, monocytes, neutrophils and eosinophils,
- neutralization of circulating autoantibodies by anti-idiotype antibodies,
- inhibition of the harmful effects due to complement activation,
- modulation of the cytokine network,
- and/or selection of immune repertoires by interaction with T and B lymphocytes.

These mechanisms may account for both the early and prolonged effects of IVIgs.

An Ig fraction (termed connected fraction) may be purified on an affinity column in which IVIg F(ab')2s or whole IgGs have been coupled to Sepharose beads (19 and 20). The IgMs contained in the serum of normal individuals bind to the F(ab')2 fragments of the autologous IgGs and inhibit the association of these IgGs with autoantigens (21). IgMs contribute to

regulating the natural autoreactivity of IgGs through interactions of the idiotypic type (21). These Igs, or their F(ab')2s, may inhibit the binding of certain autoantibodies to their antigens, as was demonstrated by tests carried out in vitro (22). The connected Ig fraction obtained from IVIgs would contain in particular antibodies which recognize anti-idiotypic determinants present on IgG or IgM autoantibodies capable of neutralizing one another and of modifying the function and dynamics of the idiotypic network (23). Moreover, an Ig fraction characterized in that it reacts with the hapten DNP is described as containing polyreactive and autoreactive natural antibodies (24).

Other documents describe the general principle for obtaining connected fractions. Among these documents, mention may be made of patent application WO 98/26086, which relates to a method for preparing a purified composition of antibodies comprising anti-idiotype antibodies, said method consisting in adsorbing a pool of IgGs onto a solid substrate containing an idiotypic determinant of an autoantibody, and in eluting.

EP 293 606 describes a general method for purifying an antibody X by idiotypic/anti-idiotypic interaction, comprising the following steps:

a) attaching an antibody Y to a solid support, said antibody recognizing the idiotype of X,
b) bringing a sample containing an antibody X into contact with the solid support in a suitable buffer,
c) eluting and d) recovering the purified antibody X.

WO 97/19113 relates to the use of monoclonal anti-idiotypic antibodies of the IgG type as immuno-regulators of the immune response, in particular for treating autoimmune diseases.

Currently, the tolerance and the effectiveness of the

polyvalent IgGs made commercially available, in particular TEGELINE® (LFB, France), are in particular recognized in the treatment of ITP, of Kawasaki disease and of retinochoroiditis of the "Birdshot" type, these being pathological conditions for which marketing authorizations have been obtained. However, the current doses in these indications are considerable and the method of administration remains laborious and complex (infusions lasting several hours in a hospital environment). The problem therefore consists in preparing a fraction which is active in autoimmune pathological conditions, so as to make the preparation more effective and more convenient to use.

The objective which is the basis of the present invention is therefore to obtain specific Igs which allow doses to be decreased, which have the same or even increased effectiveness and better tolerance, and the method of administration of which is simpler. It has been shown that it is possible to prepare fractions which address the problems mentioned above by preparing them from pools of Igs such that they have anti-IgM, anti-Ig F(ab')2 or anti-DNP reactivity and little or no reactivity with respect to non-self antigens, and/or which show polyreactivity with respect to certain autoantigens.

Description

Thus, the present invention relates to the purification of the Igs contained in the polyvalent IVIgs which are thought to be more particularly responsible for the immunomodulatory effect observed during the treatment of certain autoimmune diseases. The invention is based on the characteristics of these IgG fractions which have reactivity with respect to IgMs, IgG F(ab')2s or the hapten DNP, and little or no reactivity with respect to the tetanus toxoid and the HBs antigen (non-self antigens), i.e. fractions comprising Igs

exhibiting interactions of the idiotypic type with one another (connected fraction) or comprising natural antibodies. These fractions show polyreactivity with respect to certain autoantigens.

The Ig fractions are prepared by affinity chromatography using the property of these Igs of recognizing one another, of recognizing IgMs or of binding to the hapten DNP. The raw material used to obtain these fractions originates from polyvalent Igs, in particular those which are prepared and marketed by LFB (France), or from any other intermediate fraction obtained during the method for producing polyvalent IVIgs for therapeutic use. The general method for preparing polyvalent IVIgs essentially comprises the following steps:

- fractionation of the plasma originating from a pool of donors by precipitation, adsorption and/or filtration and then ultrafiltration (production of a first fraction, "PSO 1"),
- treatment with pepsin at acid pH, formulation, distribution and lyophilization (production of the product TEGELINE®),
- another treatment may use anion exchange column chromatography, ultrafiltration, production of an intermediate fraction (named "PSO 2") and heating, ultrafiltration, formulation and distribution (production of a liquid IVIg fraction).

In the context of the invention, the term "polyvalent Igs" is intended to mean whole polyvalent IgGs or IgMs, polyvalent IgG fragments, such as F(ab')2 or F(ab), and any intermediate fraction obtained during the method for producing polyvalent IVIgs.

A first aspect of the invention relates to an Ig fraction which reacts with at least one component selected from IgMs, IgG F(ab')2s and the hapten DNP, with a level of enrichment of greater than 20 compared

to the activity of the initial polyvalent Igs, and in that it does not react with the tetanus toxoid or the HBs antigen, with a level of enrichment of less than 5 compared to the activity of the initial polyvalent Igs.

This Ig fraction may consist of an IgG fraction or an IgM fraction.

Preferably, it reacts with a component selected from IgMs, IgG F(ab')2s and the hapten DNP, with a level of enrichment of greater than 40 compared to the activity of the initial polyvalent Igs.

The fraction according to the invention may also react with at least one of the autoantigens selected from myosin, actin, tubulin and myelin basic protein (MBP), with a level of enrichment of greater than 10, preferably 20, compared to the activity of the initial polyvalent Igs.

Advantageously, the fraction reacts with all of the autoantigens mentioned above.

A preferred fraction according to the invention may be defined in that it reacts with a component selected from IgMs, IgG F(ab')2s and the hapten DNP, with a level of enrichment of greater than 40 compared to the activity of the initial polyvalent Igs, and with myosin, actin, tubulin and MBP, with a mean level of enrichment of greater than 20 compared to the activity of the initial polyvalent Igs.

The fractions mentioned may react with IgMs or IgG F(ab')2s. They may also react with the hapten DNP and, in this case, they do not react with IgMs or IgG F(ab')2s.

A second aspect of the invention relates to a method for preparing Ig fractions, characterized in that it

comprises the following steps:

a) preparing an insoluble support onto which is grafted a component selected from polyvalent IgGs, polyvalent IgMs and DNP-lysin,

b) adsorbing polyvalent Igs onto the support obtained in step a),

c) eluting the Igs retained on the portion of immunoglobulins bound to the support, so as to collect the fraction connected through IgG-IgG or IgM-IgG idiotypic interactions, or eluting the fraction which interacts with DNP,

d) selecting the fractions having reactivity with respect to IgMs, IgG F(ab')2s or the hapten DNP, little or no reactivity with respect to non-self antigens and/or polyreactivity with respect to given autoantigens,

e) selecting the fractions having activity which inhibits the proliferation of lymphocytes in mixed culture, preferably with an effectiveness 10 to 50 times greater than TEGELINE®.

In this method, the Igs absorbed may be IgGs or IgMs.

The Ig fractions obtained are prepared from polyvalent Igs or any other intermediate fraction obtained during the method for producing IVIgs for therapeutic use. These polyvalent Igs may be IgGs or IgMs.

Within the polyvalent Igs, there are natural antibodies which interact with the hapten DNP and antibodies which interact with the idiotypes expressed by autoantibodies of the IgG or IgM type (connected fraction) and which have a certain autoreactivity. In the context of the invention, the term "connected fraction" is intended to mean a fraction which has a high percentage of Igs which interact with one another or with IgGs or IgMs via idiotype-anti-idiotype binding.

The strategy used to determine, among the various

fractions, the fraction(s) having the desired properties, i.e. the fractions which contain the highest autoreactivity titer and which react with the greatest number of autoantigens, consists in subjecting them to screening possibly comprising several successive steps.

The various in vitro and/or in vivo assays used make it possible to select, at each step, the most active fractions according to increasingly specific criteria.

The method according to the invention may therefore comprise steps for selecting Ig fractions having given characteristics.

In this sense, step d) may comprise measuring the level of enrichment of antibodies reactive against IgMs, IgG F(ab')2s or the hapten DNP used for the purification.

Step d) may also comprise measuring the reactivity for the tetanus toxoid and the HBs antigen, taking the level of enrichment as a control value.

Preferably, step d) comprises an ELISA assay carried out on a panel of autoantigens selected in particular from actin, myosin, MBP and tubulin.

Step d) of the method according to the invention may also comprise a competition assay in order to control the neutralizing activity of the fractions with respect to autoantibodies originating from serum of patients suffering from autoimmune diseases, and/or an assay of inhibition of the mixed lymphocyte reaction with human cells in order to measure the inhibitory capacity.

This mixed lymphocyte reaction assay may comprise the following steps:

- taking blood samples from a donor A and from a donor B who are incompatible in terms of major

histocompatibility complex (MHC) antigens,
- purifying the mononuclear cells on ficoll,
- culturing 2×10^5 cells from donor B in the presence of 210^5 cells from donor A,
- measuring the proliferation of the cells on day 4 by measuring tritiated thymidine incorporation.

Step a) consists in grafting polyvalent IgGs, polyvalent IgMs or DNP-Lysine onto an insoluble support, in particular onto a Sepharose®, Trisacryl®, Affiprep® or Affigel® gel, or gels activated with the groups CNBr, NHS or $C_5H_8O_2$ (glutaraldehyde). The Igs deposited onto the solid support obtained in step a) are adsorbed either in the form of polyvalent Igs lyophilized and redissolved or in liquid form, or in the form of intermediate fractions obtained during a method for producing polyvalent Igs. The Igs deposited comprise IgGs or IgMs.

Advantageously, the absorption is carried out under temperature conditions ranging from 4° to 40°C and in a 20 mM phosphate buffer or equivalent comprising NaCl, the concentration of which may range from 0 M to 3 M.

The Igs retained in step b) are preferably eluted in step c) with a buffer containing ions which dissociate Ag-Ab or DNP-Ab binding, selected in particular from chaotropes such as glycine-HCl or sodium iodide (NaI), under conditions which vary the pH, preferably between 2.8 and 4.0.

In a particular embodiment, this method comprises the following steps:

a) Grafting polyvalent IgGs, polyvalent IgMs or DNP-lysine onto a solid support or affinity (immunoadsorbant) support conventionally used in affinity chromatography. Such supports are well known to those skilled in the art. Mention may be made, for example, of a Sepharose®, Trisacryl®,

Affiprep® or Affigel® gel, or gels activated with the groups CNBr, NHS or $C_5H_8O_2$ (glutaraldehyde).

b) Adsorbing Igs in 20 mM phosphate buffer or equivalent comprising NaCl, the concentration of which may range from 0 M to 3 M, onto the solid support obtained in step a), deposited either in the form of polyvalent Igs lyophilized and redissolved or in liquid form, or in the form of intermediate fractions obtained during the method for producing polyvalent Igs. The Igs adsorbed comprise IgGs or IgMs.

c) Eluting the Igs retained in step b) with a buffer containing ions which dissociate Ag-Ac binding, selected in particular from chaotropes such as glycine-HCl or sodium iodide (NaI), under conditions which vary the pH, preferably between 2.8 and 4.0, and/or the ionic strength, and/or by any other equivalent method for breaking IgG-IgG, IgG-IgM or Ig-DNP-Lysine binding, so as to obtain Ig fractions having a reactivity profile which is different from that of the starting polyvalent Igs.

d) Measuring, by ELISA, the level of enrichment of antibodies reactive against IgMs, IgG F(ab')2s or the hapten DNP or TNP used for the purification, measuring the reactivity for the tetanus toxoid and the HBs antigen, taking the level of enrichment as a control value, and measuring the level of enrichment of reactivity with respect to a panel of autoantigens selected in particular from actin, myosin, MBP and tubulin.

As mentioned above, an additional step comprising a lymphocyte reaction assay may also be included in this method.

In each case, the fraction which is not retained on the various columns may also be used as a control in addition to the initial preparation of Ig.

Of course, certain parameters of the method may be modified at the convenience of those skilled in the art, by simple routine experiments. The invention therefore also relates to a method mentioned above, in which the parameters are determined as a function of the fractions which have been selected beforehand in step d). It involves defining the optimum parameters for obtaining a fraction having the particular properties desired and then applying these parameters on the scale of an industrial method according to the invention. Such parameters may be the parameters which characterize the fractions described above. Thus, the method may be suitable for obtaining the fractions described above. Similarly, the invention is directed toward a method for the industrial production of fractions having reactivity with respect to a component selected from IgMs, IgG F(ab')2s and the hapten DNP, little or no reactivity with respect to non-self antigens and polyreactivity with respect to given autoantigens, characterized in that steps a), b) and c) described above are carried out, respecting or adjusting the parameters used in preparing the fractions of interest selected beforehand.

The subject of the invention is also the fractions which can be obtained using the method mentioned above.

The immunomodulatory properties of the few fractions selected using the in vitro assays may also be determined in vivo in several animal models of autoimmune diseases and of graft-versus-host disease (GVH) after allografts.

Several types of model have been chosen, depending on the mechanism of action involved:

- models in which the effector function is carried out via T cells or by antibodies,
- models in which the mechanisms depend on

interaction with F(ab')2s or Fcs.

Two experimental autoimmune diseases in rats, in which the effector function is carried out via T cells, are more particularly chosen since they have been described as being sensitive to the administration of IVIgs and have the advantage of being able to provide a rapid response regarding the effectiveness of the fractions (the protective effects can be evaluated in approximately 4 weeks). They are the following models:

1) experimental autoimmune uveitis, or EAU, induced by injecting the bovine retinal antigen, or the immunodominant peptide thereof, into Lewis rats.
2) Rheumatoid arthritis (RA) induced in Dark Agouti rats by injecting bovine type II collagen.

In each case, the severity of the disease is evaluated clinically and/or histopathologically and several biological parameters, such as weight loss, production of antibodies against the autoantigen injected, are measured over time.

A model of acute GVH in rats was added since this disease has been described as being sensitive to the administration of IVIgs. GVH is induced in hybrid rats (Lewis × Brown-Norway) by injecting lymphoid cells originating from Lewis rats. The disease is evaluated by weight loss, presence of erythema and rate of mortality.

The autoimmune hemolytic anemia (AHA) animal model, which mainly involves the action of antibodies, is close to the hemolytic pathological conditions observed in humans. It is induced by injecting rat red blood cells (RBCs) into C3H mice having previously undergone a splenectomy. This assay is useful because of the effectiveness of IVIgs observed in hemolytic anemia in humans. The development of anemia is monitored via the decrease in the number of RBCs and the appearance in

the serum of the animals of autoantibodies directed against their own RBCs.

The protective effect of the product TEGELINE®, polyvalent IgGs, polyvalent IgMs or any other intermediate product obtained during the method for producing polyvalent Igs is assayed beforehand in the models and the optimum conditions for administration (dose, number, interval and route of injection) are determined. The fractions selected are injected at doses five to twenty times lower than those for TEGELINE®, and the effectiveness of these treatments is measured in the various models of autoimmune diseases.

In addition, experimental models using human cells may be used.

- The humanized SCID/NOD mouse appears to be the best model for evaluating the effectiveness in vivo on pathological human cells of the fractions preselected using the assays on animal models.

The models of primary biliary cirrhosis, of myasthenia and of Hashimoto's thyroiditis were selected since the cells originating from these pathological conditions have already been successfully transplanted into SCID mice. Other pathological conditions may subsequently be chosen.

In a subsequent phase, and with the aim of increasing knowledge regarding the mechanism of action of a given fraction, which has been demonstrated as being effective, other complementary models may be used in order to extend the indications for use of the IVIg-derived fractions, such as TEGELINE® or others.

Step d) may therefore also comprise one or more in vitro assay or assays, in particular the assays

described above.

Thus, the method according to the invention in particular allows the preparation and selection of the fractions having the characteristics defined above.

Once the fractions of interest have been identified, the parameters of steps a), b) and c) may be employed in the context of an industrial method for producing said fractions. Such a method with the suitable parameters according to the fractions of interest selected beforehand is an additional subject of the invention.

A complementary aspect of the invention relates to the fractions which can be obtained using the method defined above.

It should be noted that the description of the present invention is not limiting, and that equivalent methods and equivalent fractions also make up the invention.

The fractions according to the invention have several advantages, the main ones of which are as follows:

- A decrease in the doses. Given that the novel product provided corresponds to a fraction contained in polyvalent Igs, the amount of Ig having immunomodulatory properties which is injected is less than that of the IVIgs conventionally prescribed. The effective doses may be reduced by a factor of 5 to 20, or even more. This is a considerable advantage since the doses currently used for the available polyvalent Igs are very high: of the order of 1 to 2 g/kg.
- An effectiveness which is maintained or even increased since the product is enriched in immunomodulatory Igs.
- Better tolerance. With lower concentrations, the tolerance of the novel product is improved.

Specifically, it is currently necessary to take certain precautions when administering IVIgs with, in particular, slow infusion of the product over several hours in order to avoid certain side effects, such as for example allergic reactions.

- Simplified prescription. The administration of low doses makes it possible to instigate ambulatory treatments which substitute for the current infusions carried out in a hospital environment.

An additional aspect relates to the use of the Ig fractions according to invention, for preparing a medicinal product. This medicinal product is more particularly suitable for treating autoimmune diseases, GVH, and/or graft rejection after transplantation.

The fractions according to the invention are useful for preparing a medicinal product intended for the treatment of Kawasaki disease and/or of Birdshot retinochoroiditis, optionally in combination with corticotherapy, and/or for the treatment of certain cytopenias and/or of hemophilias with inhibitors (anti-factor VIII autoantibodies), and/or for preventing or impeding immune rejection of cell and/or organ transplants and the development of GVH after transplantation of allogenic cells.

The fractions according to the invention are also useful for preparing a medicinal product intended for the treatment of neurological diseases, in particular adult Guillain-Barré syndrome, chronic demyelinating inflammatory polyneuropathies, dermatomyositis, myasthenia and/or multiple sclerosis.

For the remainder of the description, reference will be made to the legends of the figures given hereinafter.

Legends

Figure 1A-1D: Evaluation of the properties of a fraction obtained using TEGELINE® (solid support based on Affigel grafted with TEGELINE®).
The parameters of the method for preparing this fraction are explained in greater detail in Example 1 hereinafter.
FNA means fraction not absorbed.
Figures 1A and 1C illustrate the specific reactivity with respect to IgG F(ab')2s and Figures 1B and 1D represent the reactivity with respect to autoantigens.

Figure 2A-2D: Evaluation of the properties for a fraction obtained using TEGELINE® (solid support based on NHS-Sepharose).
The parameters of the method for preparing this fraction are explained in greater detail in Example 2 hereinafter.
Figures 2A and 2C illustrate the specific reactivity with respect to IgG F(ab')2s and Figures 2B and 2D represent the reactivity with respect to autoantigens.

Figure 3A-3D: Evaluation of the properties of a fraction obtained using TEGELINE® (solid support based on NHS-AffiPrep with DNP-Lysine).
The parameters of the method for preparing this fraction are explained in greater detail in Example 3 hereinafter.
Figures 3A and 3C illustrate the specific reactivity with respect to IgG F(ab')2s and Figures 3B and 3D represent the reactivity with respect to autoantigens.

Figure 4A-4D: Evaluation of the properties of a fraction obtained using TEGELINE® (solid support based on NHS-Sepharose grafted with IgMs).
The parameters of the method for preparing this fraction are explained in greater detail in Example 4 hereinafter.

Figures 4A and 4C illustrate the specific reactivity with respect to IgMs and Figures 4B and 4D represent the reactivity with respect to autoantigens.

Figure 5: Evaluation of the capacity of TEGELINE® or of the fractions to inhibit the binding between DNA and anti-DNA antibodies originating from a serum of a patient suffering from lupus erythematosis.
The experimental conditions of the competition assay are explained in example 6 hereinafter:
■ 47-2 EN (anti-DNP); ○ 47-4 EN (anti-DNP); ❑ Tegeline; ○ 46-8 EA (anti-Tegeline); ■ 46-9 EA (anti-Tegeline).

Figure 6: Evaluation of the protective effect of the anti-DNP fraction compared to Tegeline on the development of rheumatoid arthritis induced in the rat by collagen II.
This figure represents the evolution of the arthritic score with DNP-LYSINE fractions.
The methods of induction of the disease and also of the administration of the products are described in example 7A hereinafter.

Figure 7: Evaluation of the protective effect of the anti-DNP fraction compared to Tegeline on the development of diabetes induced by cyclophosphamide in male NOD mice.
The methods of induction of the disease and also of the administration of the products are described in example 7B hereinafter.

The methods for preparing and for evaluating the activity of fractions enriched in IgGs having the property of associating with other IgGs in interactions of the idiotypic type are given in greater detail in the examples hereinafter.

Example 1: Method according to the invention with TEGELINE® and an Affigel solid support

The polyvalent IgGs were coupled to a gel made of NHS-Affigel, in a proportion of 21 mg of product per ml of gel. A dose of 20 g of polyvalent IgGs at the concentration of 20 mg/ml was brought into contact, by column recirculation, with 2 l of immunoabsorbent for 4 h at 22°C in PBS. The elution was then carried out in 0.1 M glycine-HCl, pH 3.25, and the eluate was concentrated on an ultrafiltration membrane with a cut-off threshold of 30 kD.

The concentration was measured by nephelometry. The recovery rate comes to 0.42% in the eluate and to 89% in the FNA.

The level of enrichment of reactivity with respect to F(ab')2s of this eluate compared to the starting polyvalent IgGs comes to 65.

This fraction has a reactivity which is enriched, compared to that of the polyvalent IgGs, with respect to several autoantigens and a lack of reactivity with respect to the tetanus toxoid and to the HBs antigen (see Figure 1 and Table 1).

Table 1

Antigens Tested	Level of enrichment		% recovery	
	Eluate	FNA	Eluate	FNA
F(ab')2	65	0.3	28	26
TNP	90	0.7	39	62
Toxoid	1.8	1.1	0.8	106
HBs	2.5	1.4	1.1	132
Actin	63.5	0.7	27	69
Myosin	76	0.6	33	57
MBP	29	1	12	90
Tubulin	80	0.8	34	74

Example 2: Method according to the invention with TEGELINE® and an NHS-Sepharose solid support

Polyvalent IgGs were coupled to a gel made of NHS-Sepharose, in a proportion of 10 mg of protein per ml of gel. A dose of 50 mg of polyvalent IgGs at the concentration of 1 mg/ml was brought into contact, by column recirculation, with 20 ml of immunoabsorbent for 4 h at 22°C in PBS. The fraction not absorbed, or FNA, was collected and stored at -80°C. Elution was then carried out in 0.1 M glycine-HCl buffer, pH 3.5, and the eluate was concentrated by centrifugation on an ultrafiltration membrane with a cut-off threshold of 30 kD. The IgG concentration was measured by nephelometry. The recovery rate comes to 0.77% in the eluate and to 94.7% in the FNA.

The level of enrichment of reactivity with respect to F(ab')2s of this eluate compared to the starting polyvalent IgGs comes to 76.

This fraction has a reactivity which is enriched, compared to that of the polyvalent IgGs, with respect to several autoantigens and a lack of reactivity with respect to the tetanus toxoid and to the HBs antigen (see Figure 2 and Table 2).

Table 2

Antigens tested	Level of enrichment		% recovery	
	Eluate	FNA	Eluate	FNA
TNP	32	0.45	22	39
F(ab')2	76	0.2	51	22
Toxoid	1.3	1	0.9	86
HBs	4.87	1.1	3.3	96.4
Actin	29.5	0.6	20	47
Myosin	35	0.6	24	55
MBP	29	0.7	20	58
Tubulin	22.4	0.6	15	54

Example 3: Method according to the invention with DNP-Lysine and an NHS-AffiPrep support

The DNP-Lysine was coupled to a gel made of NHS-Affiprep, in a proportion of 4 mg of product per ml of gel. A dose of 60 g of polyvalent IgGs at the concentration of 50 mg/ml was brought into contact, by column recirculation, with 2 l of immunoabsorbent for 4 h at 22°C in PBS. The elution was then carried out in 2 M sodium iodide (KI) at pH 7. After concentrating on an ultrafiltration membrane with a cut-off threshold of 30 kD, the eluate is desalified against PBS on a Sephadex G 25 column.

The concentration was measured by nephelometry. The recovery rate comes to 0.12% in the eluate and to 85% in the FNA.

The level of enrichment of reactivity with respect to TNP-Ova of this eluate compared to the starting polyvalent IgGs comes to 239.

This fraction has a reactivity which is enriched, compared to that of the polyvalent IgGs, with respect to several autoantigens and a lack of reactivity with respect to the tetanus toxoid and to the HBs antigen (see Figure 3 and Table 3).

Table 3

Antigens Tested	Level of enrichment		% recovery	
	Eluate	FNA	Eluate	FNA
TNP	239	0.9	23	94
F(ab')2	2.9	0.9	0.6	92
Toxoid	2.4	1	0.5	104
HBs	3.2	1	0.7	104
Actin	117	1.1	24	120
Myosin	83	1.2	17	129

MBP	63	1	13	102
Tubulin	137	1.5	28	152

Example 4: Method according to the invention with polyclonal IgMs and an NHS-Sepharose solid support

Human polyclonal IgMs (purity 90%) were coupled to a gel made of NHS-Sepharose, in a proportion of 10 mg of proteins per ml of gel. A dose of 50 mg of polyvalent IgGs at the concentration of 1 mg/ml was brought into contact with 20 ml of immunoadsorbent for 4 h at 22°C in PBS. The fraction not adsorbed, or FNA, was collected and stored at -80°C. The elution was then carried out in 0.1 M glycine-HCL buffer, pH 3.5, and the eluate was concentrated by centrifugation on an ultrafiltration membrane with a cut-off threshold of 30 kDa.

The IgG concentration was measured by nephelometry. The recovery rate comes to 0.20% in the eluate and to 98.7% in the FNA.

The level of enrichment of reactivity with respect to IgMs of this eluate compared to the starting polyvalent IgGs comes to 64.

This fraction has a reactivity which is enriched, compared to that of the polyvalent IgGs, with respect to several autoantigens and a lack of reactivity with respect to the tetanus toxoid and to the HBs antigen (see Figure 4 and Table 4).

Table 4

Antigens tested	Level of enrichment		% recovery	
	Eluate	FNA	Eluate	FNA
TNP	71.5	0.5	13	44.5
IgM	64	1.2	11.4	106
F(ab')2	24.5	0.7	4.5	67

Toxoid	1.8	0.8	0.3	76
HBs	< threshold	0.8	< threshold	76
Actin	52	0.3	9	33
Myosin	54	0.6	10	54
MBP	39.5	0.5	7	50
Tubulin	58	0.7	10	62

Example 5: Inhibition of the proliferation of human lymphocytes in MLC

The lymphocytes from a donor A and from a donor B which are incompatible in terms of the HLA molecules were separated on ficoll and cultured at the concentration of 2×10^5 per well in PPMI 1640 medium supplemented with 10% of fetal calf serum. Decrease in concentrations of Tegeline, of Fc or F(ab')2 fragments of Tegeline or of the various fractions given in examples 1 to 4 are added to the medium. After culturing for 4 days at 37°C in a CO_2 atmosphere, 1 µCi = 37 KBq of tritiated thymidine is added for the last 6 h of culturing. The amount of incorporation of tritiated thymidine into the human cells, which reflects the proliferation, is measured using a β scintillation counter. The percentage inhibition of the proliferation of the lymphocytes in the presence of the various components added to the culture is calculated relative to the proliferation of the mixed donor A and donor B cells. Table 5 gives the results in terms of dose in µg/ml of fractions or of products capable of giving 50% inhibition of the proliferation of the cells. The fractions given in examples 1 to 4 are capable of inhibiting the proliferation of the lymphocytes in mixed culture with an effectiveness 10 to 50 times greater than that of Tegeline.

Table 5: Inhibition by TEGELINE® and by the fractions of the proliferation of human lymphocytes in mixed culture

Reference fractions	*Affinity support*	***Dose in µg/ml giving*** *50% inhibition of the proliferation*	
		Experiment 1	***Experiment 2***
Tegeline®	NA	160	80
Fc of Tegeline®	NA	1 000	NT
F(ab')2 of Tegeline®	NA	NT	250
Example 1	AffiGel NHS Tegeline	7	NT
Example 2	Sepharose NHS Tegeline	5	NT
Example 3	AffiPrep NHS DNP-Lysine	9	2
Example 4	Sepharose NHS IgM	2.5	-

NA = not applicable
NT = not tested

Example 6: Competition assay for the fractions with respect to pathogenic antibodies

Tegeline or the anti-Tegeline fractions prepared according to example 2 or the anti-DNP fractions prepared according to example 3 are incubated, in the presence of biotinylated anti-DNA antibodies originating from a patient suffering from lupus erythematosus, in a microfiltration plate coated with DNA. The percentage inhibition of the binding of the biotinylated anti-DNA antibodies to the DNA is measured as a function of the concentration of Tegeline or of the fractions added. The results given in figure 5 show that the anti-DNP fractions inhibit the proliferation approximately ten times more than Tegiline for the same concentration. The anti-Tegeline fractions, on the

other hand, promote the binding of the pathogenic antibodies to the DNA by establishing interactions of the idiotypic type.

Example 7: Clinical applications

The fractions which are enriched in autoreactivity and which show themselves to be effective in the experimental models of autoimmune diseases are intended to be used in the treatment of many pathological conditions in which IVIgs have been shown to a have a therapeutic action, and in particular autoimmune diseases, GVH and graft rejection after transplantation.

Example 7A: Effect of the anti-DNP fractions compared to Tegeline on the development of rheumatoid arthritis induced in the rat by collagen II.

The fractions enriched in autoreactivity which originate from the elution of polyvalent IgGs from a gel made of NHS-Affiprep coupled to DNP-Lysine (fig. 3 and example 3) were injected *ip* at various doses into rats which had been given collagen II to induce the development of rheumatoid arthritis. The effectiveness of protection against rheumatoid arthritis of the fractions was compared to that obtained with the same doses of initial polyvalent IgGs. The cumulated results of two independent experiments (figure 6) show that the dose effective on the development of rheumatoid arthritis of the fractions originating from the elution of the gel made of NHS-Affiprep coupled to DNP-Lysine is ten times less than the effective dose of Tegeline.

Example 7B: Effect of the anti-Tegeline fraction and of the anti-DNP fraction on the development of diabetes induced by cyclophosphamide in male NOD mice.

Newborn male NOD mice are injected three times a week

for four weeks either with Tegeline at the dose of 1 mg/young mouse, or with the anti-Tegeline fraction or anti-DNP fraction at the dose of 0.1 mg/young mouse. The development of diabetes is triggered at 8 weeks old by two injections of cyclophosphamide (200 mg/kg) two weeks apart. Figure 7 shows that the percentage of diabetic mice (level of sugar in blood greater than 3g/l) is significantly decreased in the group of NOD mice injected with Tegeline (14%) and in the group injected with the anti-DNP fraction (21%), but not in the group injected with the anti-Tegeline fraction, compared to the nontreated group (68%).

These indications are not exclusive and may be extended. Said fractions are formulated with a pharmaceutical vehicle suitable for intravenous administration, with packaging either in lyophilized form or in liquid form, or another route (IP, ID, IM), depending on the desired indications.

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CLAIMS

1. An Ig fraction, characterized in that it reacts with at least one component selected from IgMs, IgG F(ab')2s and the hapten DNP, with a level of enrichment of greater than 20 compared to the activity of the initial polyvalent Igs, and in that it does not react with the tetanus toxoid or the HBs antigen, with a level of enrichment of less than 5 compared to the activity of the initial polyvalent Igs.

2. The Ig fraction as claimed in claim 1, characterized in that it consists of an IgG or IgM fraction.

3. The fraction as claimed in either of claims 1 and 2, characterized in that it reacts with a component selected from IgMs, IgG F(ab')2s and the hapten DNP, with a level of enrichment of greater than 40 compared to the activity of the initial polyvalent Igs.

4. The fraction as claimed in one of claims 1 to 3, characterized in that it reacts with at least one of the autoantigens selected from myosin, actin, tubulin and myelin basic protein (MBP), with a level of enrichment of greater than 10 compared to the activity of the initial polyvalent Igs.

5. The fraction as claimed in claim 4, characterized in that it reacts with at least one of the autoantigens selected from mysoin, actin, tubulin and MBP, with a level of enrichment of greater than 20 compared to the activity of the initial polyvalent Igs.

6. The fraction as claimed in one of claims 1 to 5,

characterized in that it reacts with myosin, actin, tubulin and MBP.

7. The fraction as claimed in one of claims 1 to 6, characterized in that it reacts with a component selected from IgMs, IgG F(ab')2s and the hapten DNP, with a level of enrichment of greater than 40 compared to the activity of the initial polyvalent Igs, and with myosin, actin, tubulin and MBP, with a mean level of enrichment of greater than 20 compared to the activity of the initial polyvalent Igs.

8. The fraction as claimed in one of claims 1 to 7, characterized in that it reacts with IgMs or IgG F(ab')2s.

9. The fraction as claimed in one of claims 1 to 7, characterized in that it reacts with the hapten DNP and in that it does not react with IgMs and IgG F(ab')2s.

10. A method for preparing Ig fractions, characterized in that it comprises the following steps:

a) preparing an insoluble support onto which is grafted a component selected from polyvalent IgGs, polyvalent IgMs and DNP-lysin,

b) adsorbing polyvalent Igs onto the support obtained in step a),

c) eluting the Igs retained on the portion of immunoglobulins bound to the support, so as to collect the fraction connected through IgG-IgG or IgM-IgG idiotypic interactions, or eluting the fraction which interacts with DNP,

d) selecting the fractions having reactivity with respect to IgMs, IgG F(ab')2s or the hapten DNP, little or no reactivity with respect to non-self antigens and/or polyreactivity with respect to given autoantigens,

e) selecting the fractions having activity which inhibits the proliferation of lymphocytes in mixed culture, preferably with an effectiveness 10 to 50 times greater than TEGELINE®.

11. The method as claimed in claim 10, characterized in that the Igs absorbed consist of IgGs or IgMs.

12. The method as claimed in either of claims 10 and 11, characterized in that the Ig fractions are prepared from polyvalent Igs or any other intermediate fraction obtained during the method for producing IVIgs for therapeutic use.

13. The method as claimed in claim 12, characterized in that the polyvalent Igs used to prepare the fractions consist of IgGs or IgMs.

14. The method as claimed in one of claims 10 to 13, characterized in that step d) comprises measuring the level of enrichment of antibodies reactive against IgMs, IgG F(ab')2s or the hapten DNP used for the purification.

15. The method as claimed in one of claims 10 to 14, characterized in that step d) comprises measuring the reactivity for the tetanus toxoid and the HBs antigen, taking the level of enrichment as a control value.

16. The method as claimed in one of claims 10 to 15, characterized in that step d) comprises an ELISA assay carried out on a panel of autoantigens selected in particular from actin, myosin, MBP and tubulin.

17. The method as claimed in one of claims 10 to 16, characterized in that step d) comprises a competition assay in order to control the

neutralizing activity of the fractions with respect to autoantibodies originating from serum of patients suffering from autoimmune diseases.

18. The method as claimed in one of claims 10 to 17, characterized in that step d) comprises an assay of inhibition of the mixed lymphocyte reaction with human cells in order to control the reactivity of the purified Igs.

19. The method as claimed in one of claims 10 to 18, characterized in that step a) consists in grafting polyvalent IgGs, polyvalent IgMs or DNP-Lysine onto an insoluble support, in particular onto a Sepharose®, Trisacryl®, Affiprep® or Affigel® gel, or gels activated with the groups CNBr, NHS or $C_5H_8O_2$ (glutaraldehyde).

20. The method as claimed in one of claims 10 to 19, characterized in that the Igs deposited onto the solid support obtained in step a) are adsorbed either in the form of polyvalent IgGs lyophilized and redissolved or in liquid form, or in the form of intermediate fractions obtained during a method for producing polyvalent IgGs, in 20 mM phosphate buffer containing NaCl, the concentration of which may range from 0 M to 3 M.

21. The method as claimed in one of claims 10 to 20, characterized in that the Igs retained in step b) are eluted with a buffer containing ions which dissociate Ag-Ab or Ag-DNP binding, selected in particular from chaotropes such as glycine-HCl or sodium iodide (NaI), under conditions which vary the pH, preferably between 2.8 and 4.0, and/or the molarity of the buffer.

22. The method as claimed in one of claims 10 to 21, characterized in that the absorption is carried

out under temperature conditions ranging from 4° to 40°C and in PBS.

23. The method as claimed in one of claims 10 to 22, characterized in that, in step d), fractions as claimed in one of claims 1 to 9 are selected.

24. A method for the industrial production of fractions having reactivity with respect to a component selected from IgMs, IgG F(ab')2s and the hapten DNP, little or no reactivity with respect to non-self antigens and polyreactivity with respect to given autoantigens, characterized in that steps a), b) and c) of claim 10 are carried out, respecting or adjusting the parameters used in preparing the fractions of interest selected beforehand.

25. A fraction which can be obtained using a method as claimed in one of claims 10 to 24.

26. The use of an Ig fraction as claimed in one of claims 1 to 9 and 25, for preparing a medicinal product.

27. The use as claimed in claim 26, for preparing a medicinal product intended for the treatment of autoimmune diseases, or GVH and/or of graft rejection after transplantation.

28. The use as claimed in claim 26, for preparing a medicinal product intended for the treatment of Kawasaki disease, for the treatment of Birdshot retinochoroiditis, optionally in combination with corticotherapy, and/or for the treatment of certain cytopenias and/or of hemophilias with inhibitors (anti-factor VIII autoantibodies), and/or for preventing and/or impeding immune rejection of cell and/or organ transplants and the

development of GVH after transplantation of allogenic hematopoietic cells.

29. The use as claimed in claim 26, for preparing a medicinal product intended for the treatment of neurological diseases, in particular adult Guillain-Barré syndrome, chronic demyelinating inflammatory polyneuropathies, dermatomyositis, myasthenia and/or multiple sclerosis.

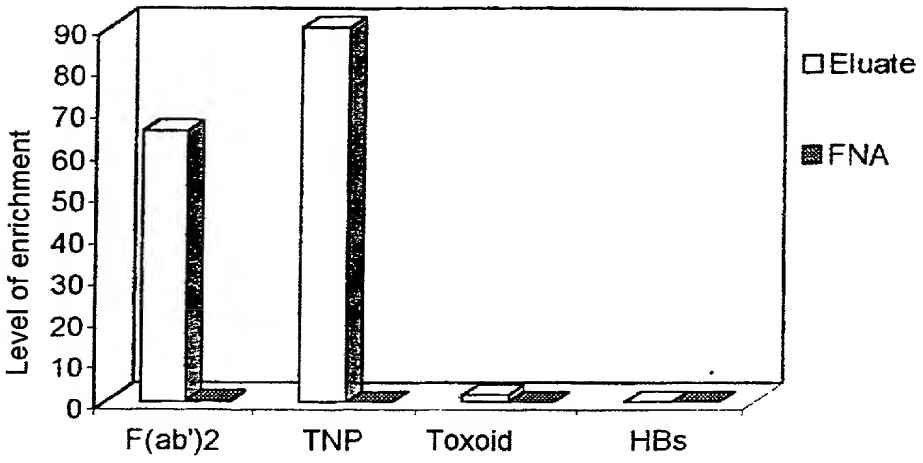


FIGURE 1A

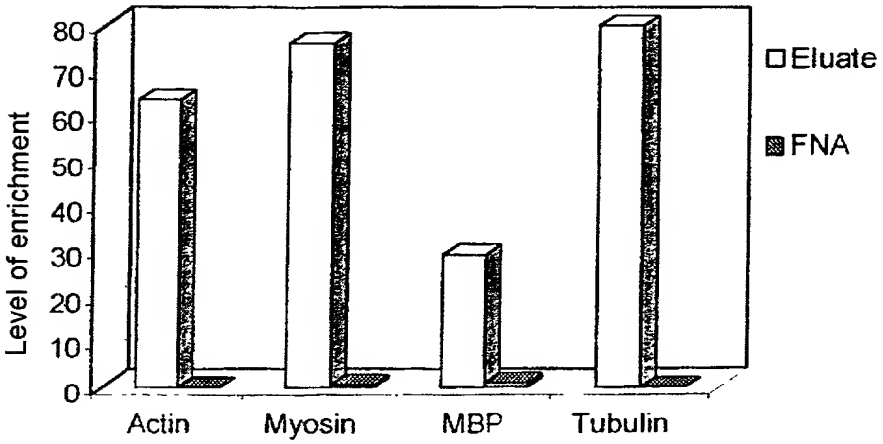


FIGURE 1B

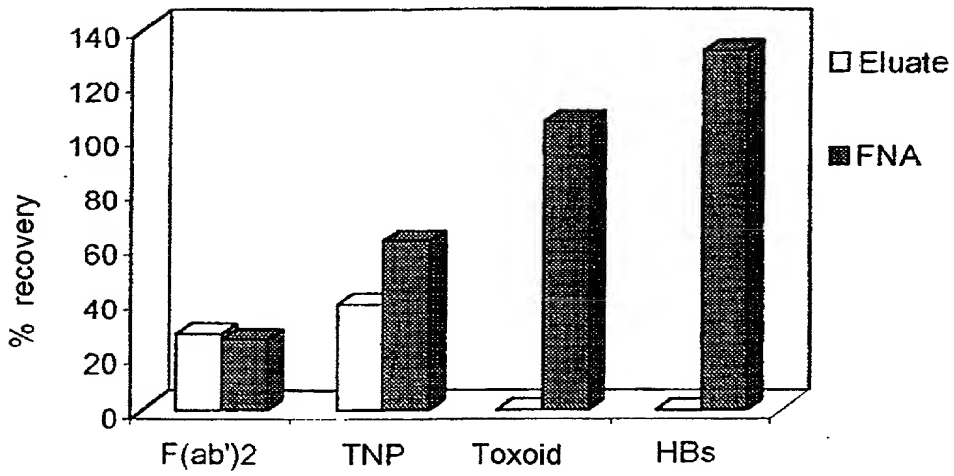


FIGURE 1C

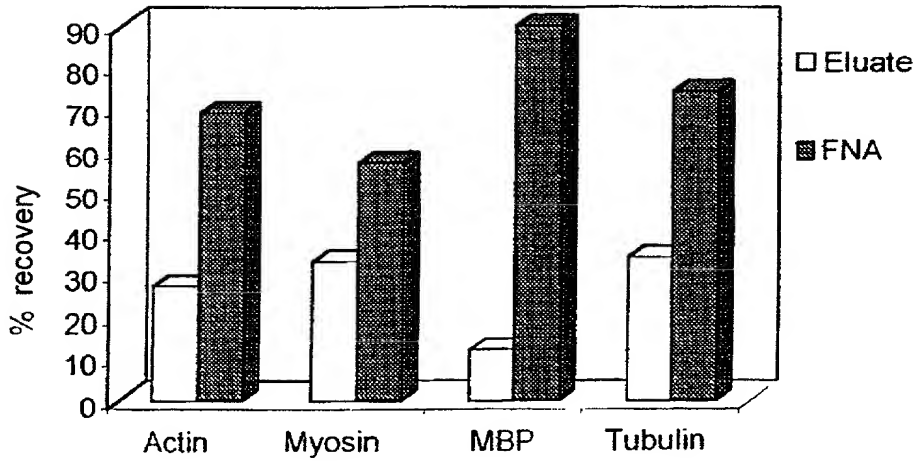


FIGURE 1D

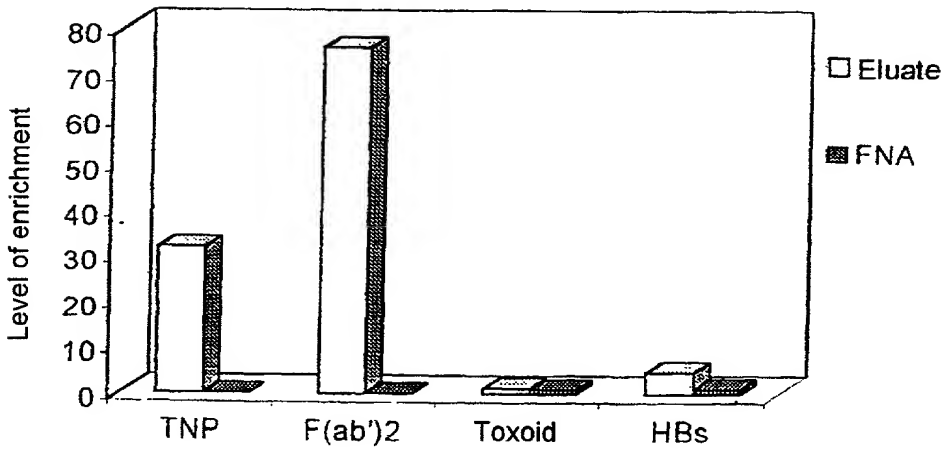


FIGURE 2A

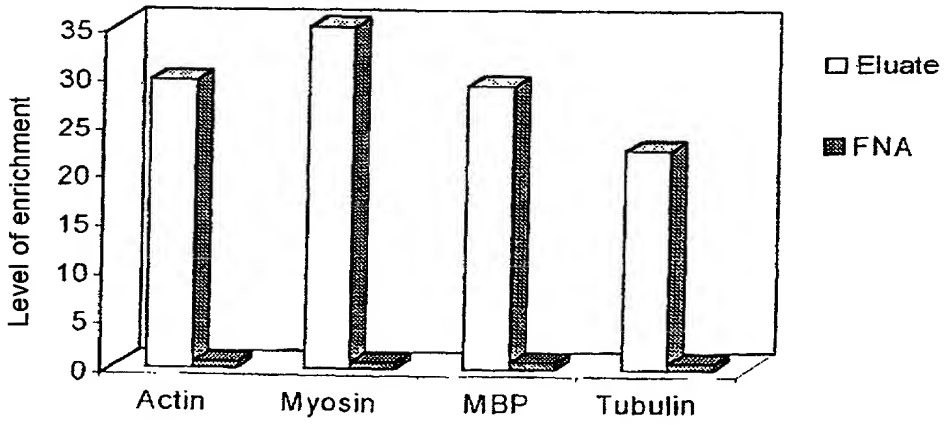


FIGURE 2B

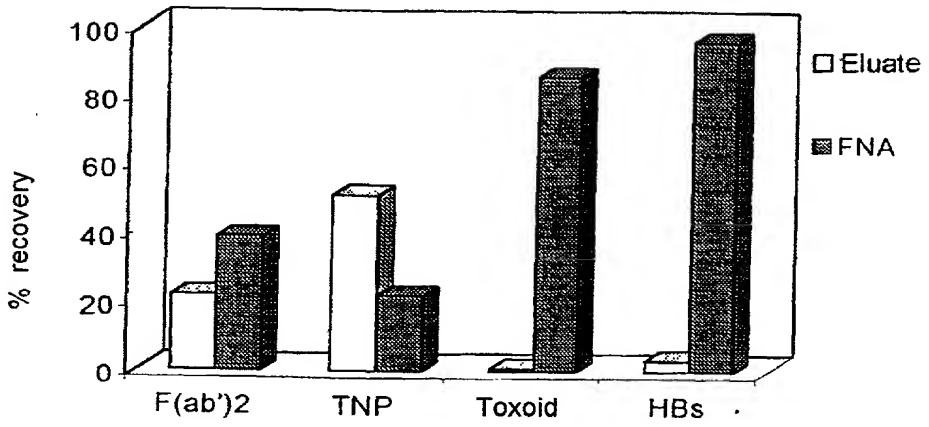


FIGURE 2C

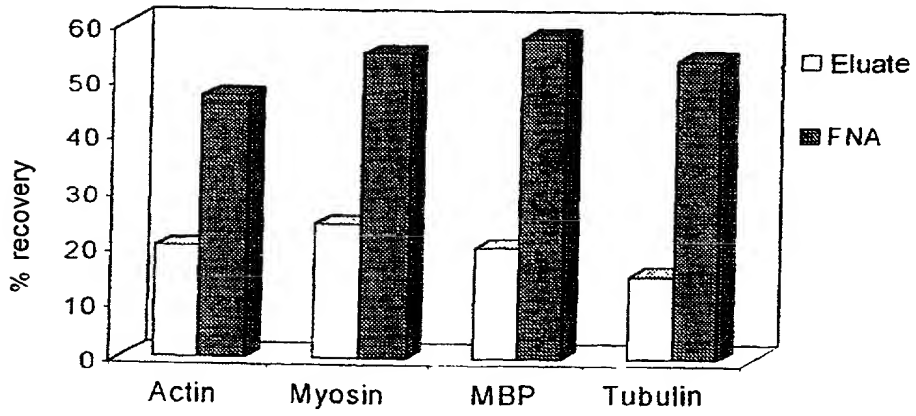


FIGURE 2D

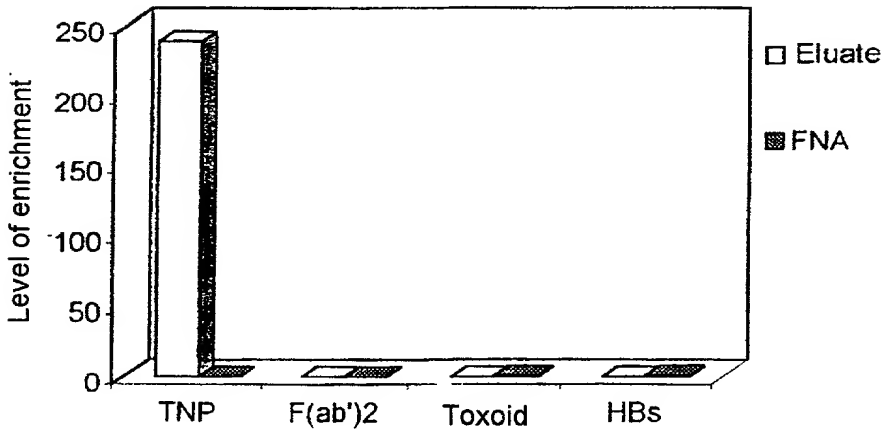


FIGURE 3A

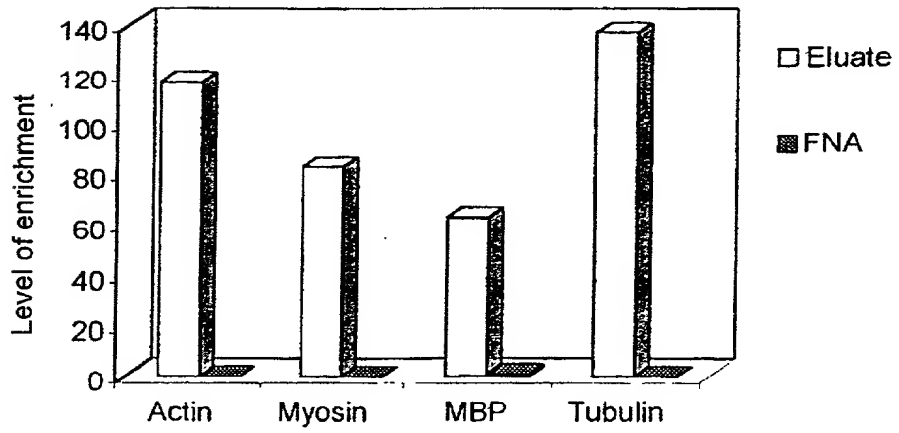


FIGURE 3B

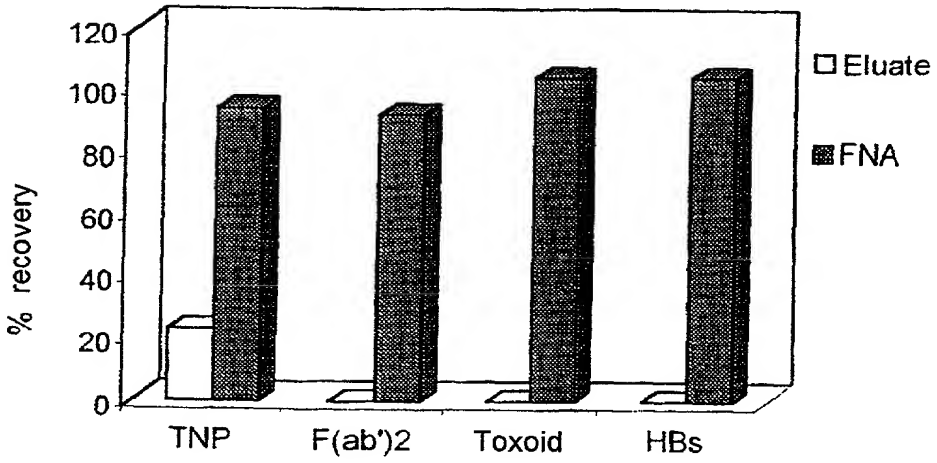


FIGURE 3C

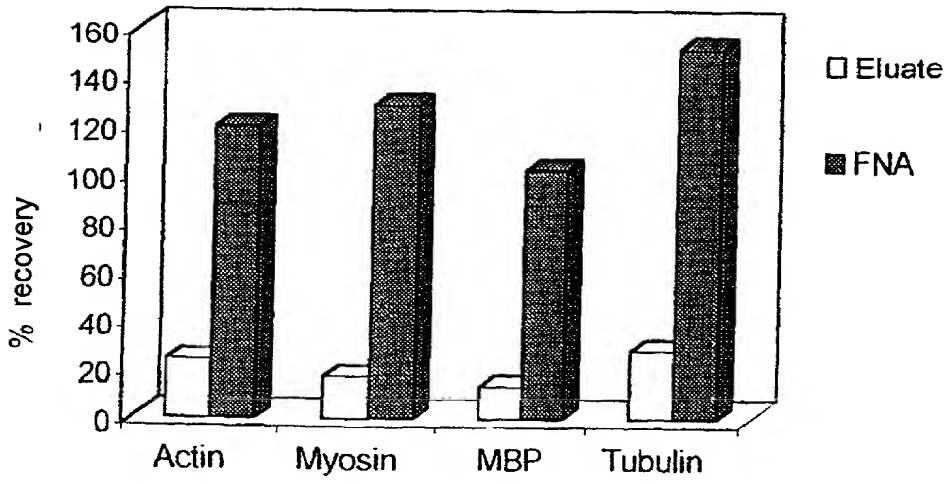


FIGURE 3D

FIGURE 4A

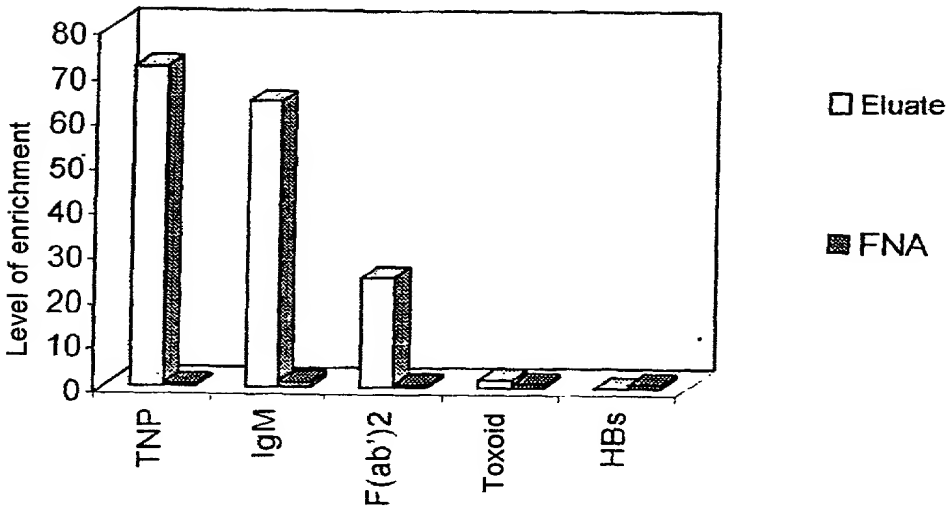


FIGURE 4B

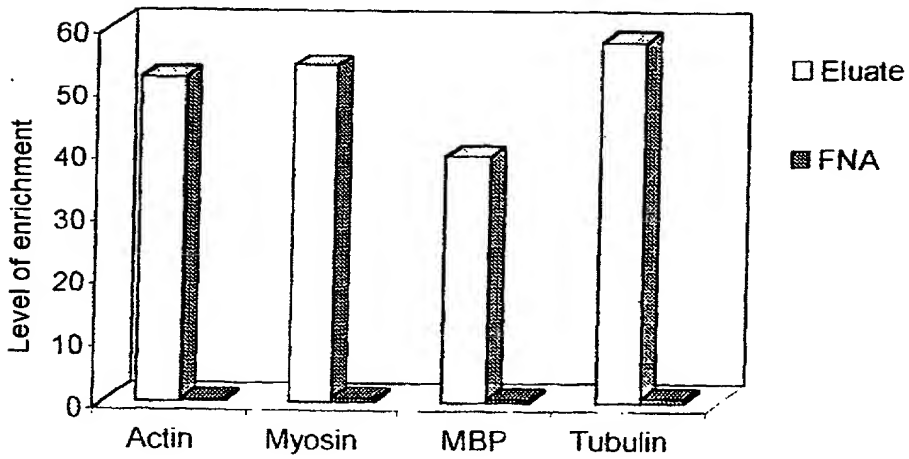


FIGURE 4C

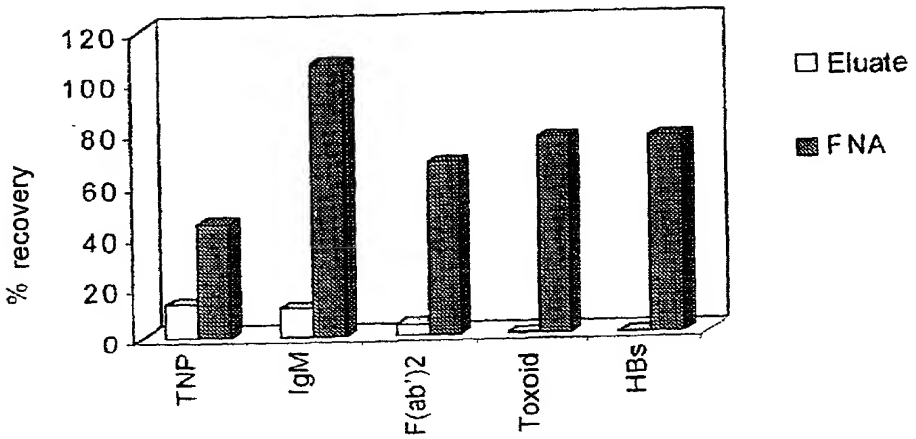
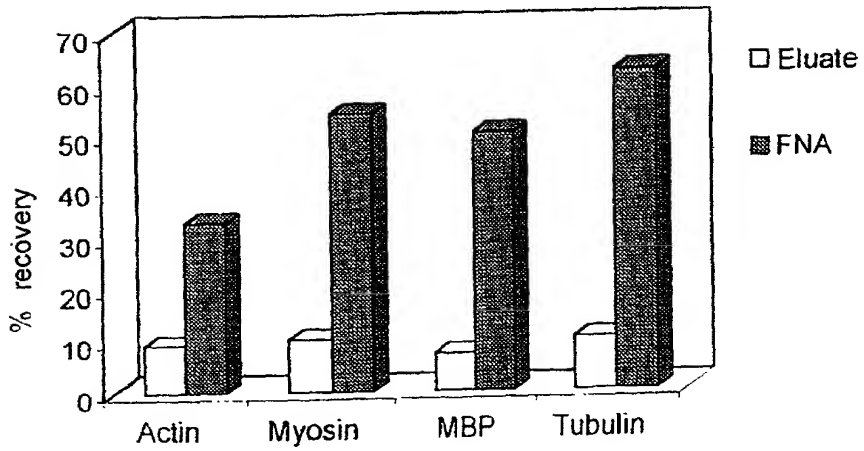


FIGURE 4D



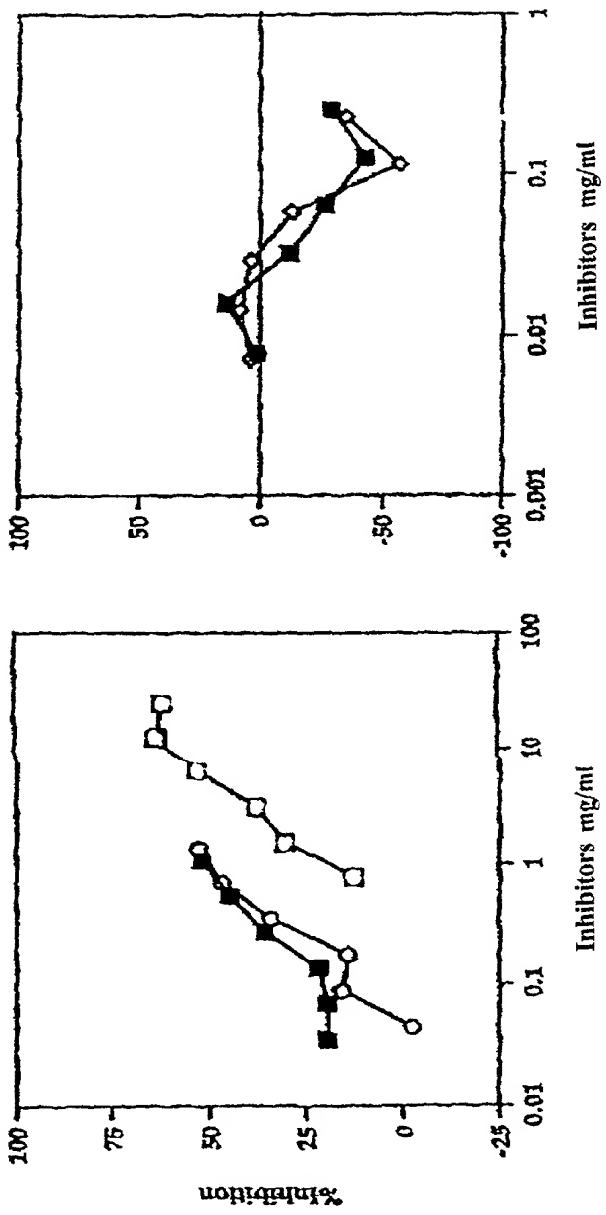

100
50
0
-50
-100
0.001
0.01
0.1
1
Inhibitors mg/ml
100
75
50
25
0
-25
0.01
0.1
1
10
100
Inhibitors mg/ml
%Inhibition

FIGURE 5

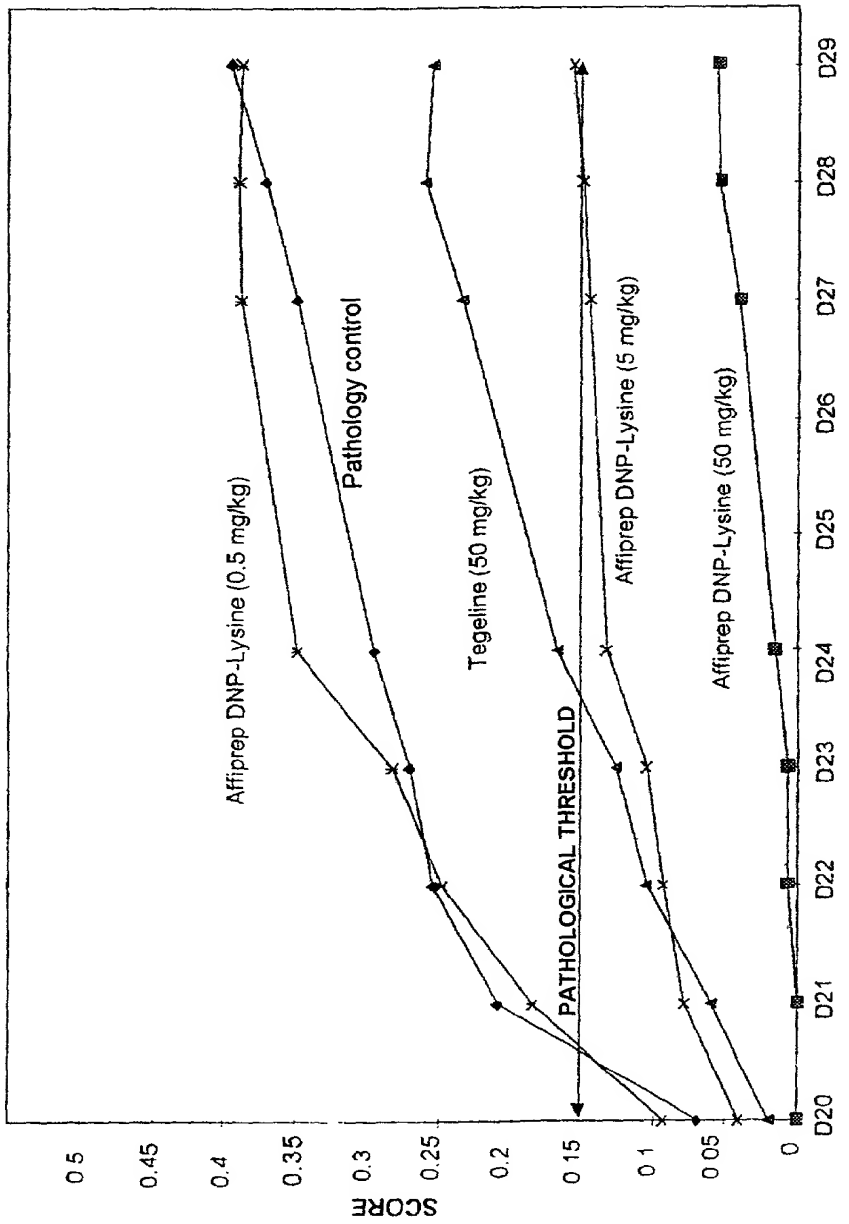

SCORE
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
D20
D21
D22
D23
D24
D25
D26
D27
D28
D29
DAY
Affiprep DNP-Lysine (0.5 mg/kg)
Pathology control
Tegeline (50 mg/kg)
PATHOLOGICAL THRESHOLD
Affiprep DNP-Lysine (5 mg/kg)
Affiprep DNP-Lysine (50 mg/kg)

FIGURE 6

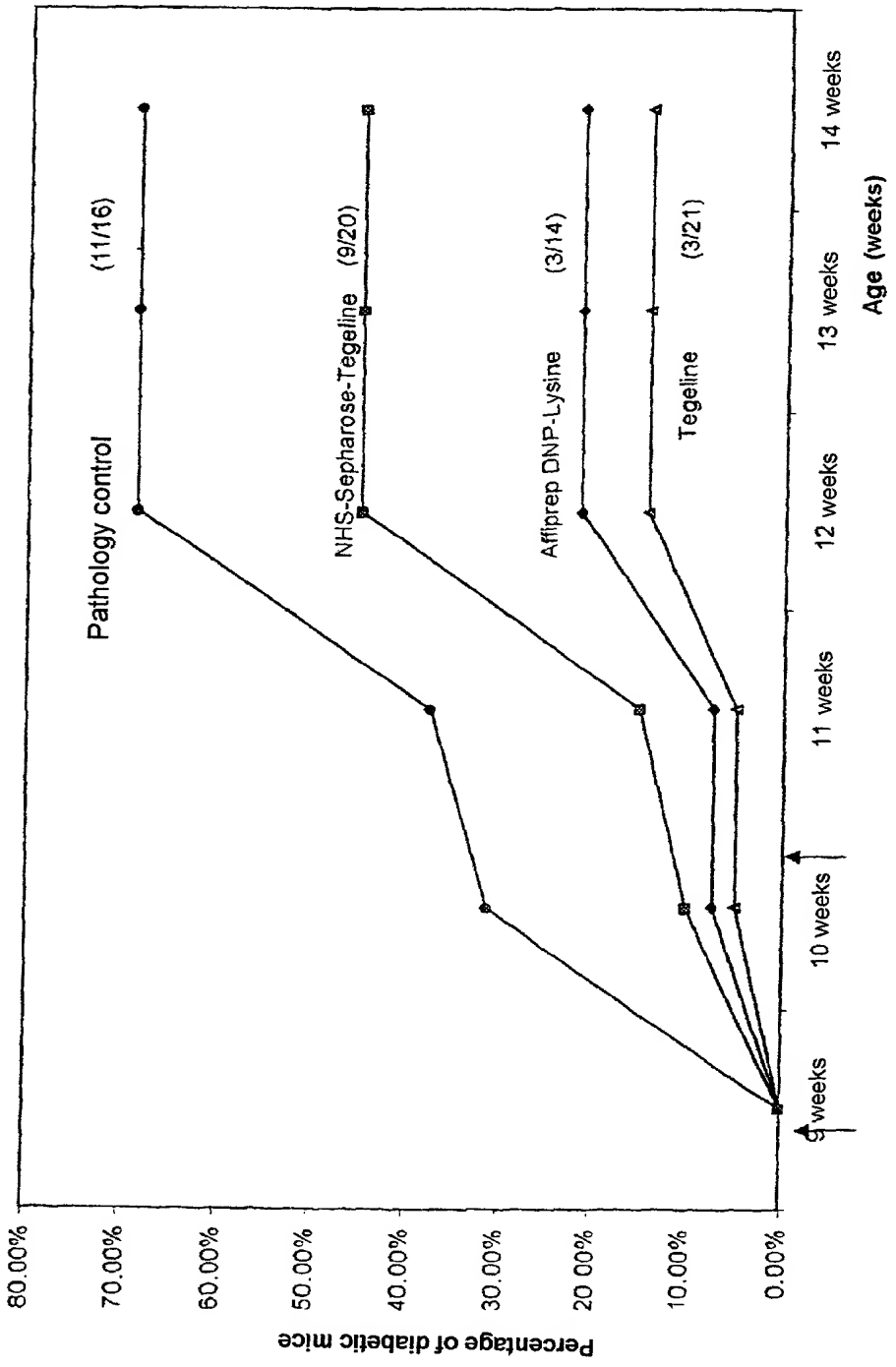

Percentage of diabetic mice
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
9 weeks
10 weeks
11 weeks
12 weeks
13 weeks
14 weeks
Age (weeks)
Pathology control (11/16)
NHS-Sepharose-Tegeline (9/20)
Affiprep DNP-Lysine (3/14)
Tegeline (3/21)

FIGURE 7

Docket No. _____

DECLARATION AND POWER OF ATTORNEY

#3

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:.

NOVEL iG FRACTIONS HAVING IMMUNOMODULATORY ACTIVITY

the specification of which is attached hereto unless the following box is checked:

☒ was filed on June 7, 2000 as ~~United States Application Number or~~ PCT International Application Number PCT/FR00/01560 and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN APPLICATION(S)

NUMBER	COUNTRY	DAY/MONTH/YEAR FILED	PRIORITY CLAIMED
99 07153	France	07/06/1999	XX
99 16632	France	29/12/1999	XX

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

APPLICATION NO.	FILING DATE

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

APPLICATION SERIAL NO.	FILING DATE	STATUS: PATENTED, PENDING, ABANDONED
PCT/FR00/01560	07.06.2000	pending

19) I hereby appoint as my attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: Stephen A. Bent, Reg. No. 29,768; David A. Blumenthal, Reg. No. 26,257; William T. Ellis, Reg. No. 26,874; John J. Feldhaus, Reg. No. 28,822; Patricia D. Granados, Reg. No. 33,683; John P. Isacson, Reg. No. 33,715; Donald D. Jeffery, Reg. No. 19,980; Eugene M. Lee, Reg. No. 32,039; Richard Linn, Reg. No. 25,144; Peter G. Mack, Reg. No. 26,001; Brian J. McNamara, Reg. No. 32,789; Sybil Meloy, Reg. No. 22,749; George E. Quillin, Reg. No. 32,792; Colin G. Sandercock, Reg. No. 31,298; Bernhard D. Saxe, Reg. No. 28,665; Charles F. Schill, Reg. No. 27,590; Richard L. Schwaab, Reg. No. 25,479; Arthur Schwartz, Reg. No. 22,115; Harold C. Wegner, Reg. No. 25,258.

Docket No._____

Address all correspondence to FOLEY & LARDNER, Washington Harbour, 3000 K Street, N.W., Suite 500, P.O. Box 25696, Washington, D.C. 20007-8696. Address telephone communications to _____ at (202) 672-5300.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1-00

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Residence Address		Country of Citizenship
Post Office Address		